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Application of: May et al.

Application No.: 10/721,001

Group Art Unit: to be assigned

Filed: November 25, 2003

Examiner: unassigned

For: A METHOD OF EXTRACTING AND
ISOLATING MINOR COMPONENTS
FROM VEGETABLE OIL

Attorney Docket No.: 061255-0028
(previously 3456-028)

SUBMISSION OF PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Under the provisions of 35 U.S.C. § 119, Applicants hereby claim the benefit of the filing date of Malaysian Application No. PI 2002 4432, filed November 27, 2002 for the above-identified United States Patent Application. In support of Applicants' claim for priority, filed herewith is one certified copy of the above.

No fee is believed to be due for this submission. If any fees are due, however, please charge the required fees to Morgan, Lewis & Bockius LLP Deposit Account No. 50-0310.

Respectfully submitted,

Date: April 26, 2004

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PATENT APPLICATION NO: PI 2002 4432

This is to certify that annexed hereto is a true copy from the records of the Registry of Trade Marks and Patents, Malaysia of the application as originally filed which is identified therein.

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(CERTIFYING OFFICER)
12 January 2004



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CERTIFICATE OF FILING

APPLICANT : MALAYSIAN PALM OIL BOARD
APPLICATION NO : PI 20024432
REQUEST RECEIVED ON : 27/11/2002
FILING DATE : 27/11/2002
AGENT'S/APPLICANT'S FILE REF. : SD/PAT/2459782/ZRS/CD

Please find attached, a copy of the Request Form relating to the above application, with the filing date and application number marked thereon in accordance with Regulation 25(1).

Date : 29/11/2002


.....
(ROZILEE BIN ASID)
for Registrar of Patents

To : WONG SAI FONG
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Patents Form 1
PATENTS ACT 1983

**REQUEST FOR
GRANT OF PATENT**
[Regulation 7(1)]

To: The Registrar of Patents
Patent Registration Office
Kuala Lumpur, Malaysia

FOR OFFICIAL USE

Application No. A 2002-4432
Filing date: 27-11-2002
Request received on: 27-11-2002
Fee received on: 27-11-2002
Amount: RM 570
Cheque No. 200 329049

*Please submit this Form in duplicate together
with the prescribed fee.*

Applicant or Agent's file reference:
SD/PAT/2459782/ZRS/CD

THE APPLICANT(S) REQUEST(S) THE GRANT OF A PATENT IN RESPECT OF THE
FOLLOWING PARTICULARS.

I. TITLE OF INVENTION

A METHOD OF EXTRACTING AND ISOLATING MINOR COMPONENTS
FROM VEGETABLE OIL

II. APPLICANT(S)

*(The data concerning each applicant must appear in this box or, if the space is insufficient,
in the space below)*

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*Permanent residence or
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Additional Information

*Delete whichever does not apply

20024432

III. INVENTOR(S)

The Applicant(s) is/are the inventor(s):

Yes ☐

No ☒

If the Applicant(s) is/are not the inventor(s):

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A statement justifying the applicant's right to the patent
accompanies this form:

Yes

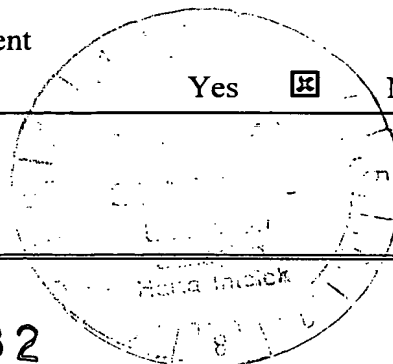


No



Additional information

20024432



IV. AGENT OR REPRESENTATIVE

The Applicant(s) has/have appointed a patent agent in the accompanying Form 17 (will follow) Yes ☒ No ☐

Patent Agent's Registration number: PA 86/0003 and/or 86/0004

Applicants have appointed to be their common representative.

V. DIVISIONAL APPLICATION

This application is a divisional application: ☐

The benefit of the filing date ☐ and priority date ☐ of the initial application is claimed inasmuch as the subject matter of the present application is contained in the initial application identified below:

Initial Application number:

Date of filing of initial application:

VI. DISCLOSURES TO BE DISREGARDED FOR PRIOR ART PURPOSES

Additional information is contained in supplemental box:

(a) Disclosure was due to acts of the applicant or his predecessor-in-title ☐

Date of disclosure:

(b) Disclosure was due to the abuse of rights of applicant or his predecessor-in-title ☐

Date of disclosure:

A statement specifying in more detail the facts concerning the disclosure Yes ☐

Accompanies this Form No ☐

Additional information (if any)

VII. PRIORITY CLAIM (if any)

The priority of earlier applicant(s) is/are claimed as follows:-

<u>Country*</u>	<u>Application no.</u>	<u>Filing date (dd/mm/yy)</u>
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Nil

Nil

Nil

Symbol of the International Patent Classification:

If not yet allocated, please tick

The priority of more than one earlier application is claimed: Yes ☐ No ☐

The certified copy of the earlier application(s) accompanies this form: Yes ☐ No ☐

If No, it will be furnished upon request.

*if the earlier application is a regional or international application, indicate the office with which it is filed

20024432

Additional information (if any)

VII. CHECK LIST

A. This application contains the following:

1.	Request (Form 1)	04	sheets
2.	description	19	sheets
3.	claim(s)	05	sheets
4.	abstract	01	sheets
5.	drawings (if any)	-	sheets
TOTAL		29	

B. This Form, as filed, is accompanied by the items checked below:

- | | | |
|----|---|-------------------------------------|
| a. | signed Form No. 17 (will follow) | <input checked="" type="checkbox"/> |
| b. | declaration that inventor does not wish to be named in the patent | <input type="checkbox"/> |
| c. | statement justifying applicant's right to the patent | <input checked="" type="checkbox"/> |
| d. | statement that certain disclosure be disregarded | <input type="checkbox"/> |
| e. | priority document (certified copy of earlier application) | <input type="checkbox"/> |
| f. | cheque, cash, etc. for the payment of application fee | <input checked="" type="checkbox"/> |
| g. | other documents (specify) | <input type="checkbox"/> |

IX. SIGNATURE

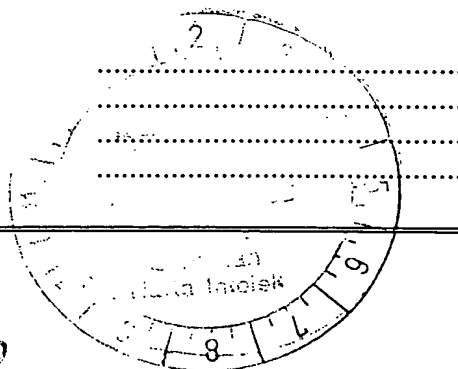


.....
Name: WONG SAI FONG
Regn No.: PA 86/0003

27 November 2002
.....
date

For official use:

1. Date application received:
2. Date of receipt of
correction,
later filed papers, or
drawings
completing the application.



20024432

A METHOD OF EXTRACTING AND ISOLATING MINOR COMPONENTS FROM VEGETABLE OIL

FIELD OF THE INVENTION

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The present invention relates to a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil.

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BACKGROUND OF THE INVENTION

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Crude palm oil (CPO) contains about 1% minor components which include carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols. These minor components are also present in oil that is obtained from the palm pressed fiber in a much higher concentration.

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Carotenes, tocols and squalene have been known for long to exhibit antioxidant properties and they are widely used in pharmaceuticals as supplements, nutraceuticals as well as fine chemicals. Squalene, which is widely found in shark liver oil is also present in palm oil and it is a valuable constituent in cosmetics as well as food supplements. Sterols can be used as steroid derivatives in pharmaceuticals. The major sterol present in palm oil, β -sitosterol has been shown to possess hypocholesterolemic effect.

Several methods have been developed to extract these valuable minor components from vegetable oils namely, extraction by saponification which have been disclosed in US Patents No. 2,460,796, 2,572,467 and 2,652,433 as well as in UK Patent No. GB 567,682, iodine method, urea process and extraction by Fuller's earth or activated
5 carbon which have been disclosed in UK Patents No. 691,924 and 1,563,794 as well as in US Patent 2,484,040.

Further extraction methods which are known to artisans in the field of the invention are solvent extraction which has been disclosed in US Patent 2,432,021, molecular
10 distillation, solvent partitioning and adsorption which has disclosed in US Patent 5,157,132 and UK Patent No. 2,160,874.

SUMMARY OF THE INVENTION

The present invention relates a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil. The said method process comprises the steps of a) esterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components, b) collection of the esters phase containing the minor components from glycerol, c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr, d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar, e) adsorption of the concentrate obtained from step (c) on an adsorbent, f) extraction of the minor components from the concentrate obtained from step (e) by desorption of the minor components using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar and g) desorption of the minor components by a predetermined mixture of solvents.

20

There is also provided a method for isolating individual carotene such as β -carotene, α -carotene, lycopene, phytoene and phytofluene, individual tocol such as α -, γ - and δ -tocopherol and tocotrienol and individual sterol such as β -sitosterol from vegetable oil under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the

method comprising the steps of a) adsorbing the oil to an adsorbent and b) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

Also provided a method for isolating individual carotene such as β -carotene, α -carotene, lycopene, phytoene and phytofluene, individual tocol such as α -, γ - and δ -tocopherol and tocotrienol and individual sterol such as β -sitosterol from minor components such as squalene, carotenes, tocols and sterols obtained from a method which is conducted under an isocratic condition, the method comprising the steps of a) esterification/transesterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components, b) collection of the esters phase containing the minor components from glycerol, c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr, d) dissolution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar, e) adsorption of the concentrate obtained from step (c) on an adsorbent, f) extraction of the minor components from the concentrate obtained from step (e) using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar and g) desorption of the minor components by a predetermined mixture of solvents characterised in that the minor components obtained from the above method undergo a further extraction under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the extraction comprising

the steps of 1) adsorbing the minor components to an adsorbent and 2) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

The present invention consists of certain novel features and a combination of parts
5 hereinafter fully described and particularly pointed out in the appended claims, it being understood that various changes in the details may be without departing from the scope of the invention, or sacrificing any of the advantage of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a method of extracting and isolating minor components such as carotenes, tocols in the form of tocopherols and tocotrienols and hydrocarbons such as squalene and phytosterols from vegetable oils, preferably but not necessarily from palm oil. Hereinafter, this specification will describe the present invention according to the preferred embodiments. However, it is to be understood that limiting the description to the preferred embodiments of the invention is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications and equivalents without departing from the scope of the appended claims.

The vegetable oil is esterified with lower alcohols such as methanol, ethanol, isopropanol and butanol to give a mixture of glycerol, fatty acids alkyl esters, carotenes, tocols, squalene and sterols. The minor components are contained in the oil phase of the mixture which is then separated from glycerol.

The esters phase containing minor components is subjected to distillation under or without vacuum in which it is heated to not more than 200⁰C and distilled at a pressure less than 150 mm Torr to retain as much of the minor components in the concentrate. Upon distillation, the fatty acid alkyl esters are removed from the mixture, leaving a concentrate rich in carotenes, tocols, squalene and sterols.

The concentrate collected is dissolved in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with the non-polar solvent being the major constituent and adsorbed on a selected adsorbent. Different types of adsorbents such as normal-phase silica gel, reversed-phase (particularly C18) silica gel or neutral alumina may be employed. The minor components are extracted and desorbed from the adsorbents by a certain mixture of solvents depending on the type of adsorbent in use. Solvents that are used to desorb as well as to carry the minor components may consist of polar solvents such as ethanol, isopropanol, methanol or butanol, non-polar solvents such as hexane, heptane, ethyl acetate, isooctane or cyclohexane.

10

The extraction or fractionation is carried out in an isocratic manner which is an improvement over US Patent No. 6,072,092 and UK Patent No. GB 2218989 whereby solvents with different polarity are added one after another to desorb all the compounds which is rather time and labour consuming, most important of all it is not technically and economically viable for commercial production. Also, US Patent No. 6,072,092 has only disclosed recovery of carotenes from palm oil in all the examples whereas the present invention disclose recovery of carotene, vitamin E, sterols and squalene in a single adsorption/desorption process.

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The desorption of the minor components from the adsorbents is carried out through a column whereby it is subjected to an applied pressure between 0.2 to 50 bar resulting in faster and more efficient separation.

The above-described process can also be adopted for the isolation of individual carotene (i.e. β -carotene, α -carotene, lycopene, phytoene, phytofluene), tocol (α -, γ - and δ -tocopherol and tocotrienol) and sterol (β -sitosterol) from vegetable oils. Isolation of such individual compounds is an extension of the first extraction or from any sources that are rich in carotenes, tocols or sterols wherein operating pressure of the said process may be in the range of 0.2 – 1000 bar. Isobaric and isocratic conditions are applied.

Each resulting fractions are then subjected to a second extraction wherein individual carotene (α -carotene, β -carotene, lycopene, phytoene, phytofluene), tocol (α -, γ - and δ -tocopherol and tocotrienol) and β -sitosterol can be isolated and recovered. The second method consists of adsorbing the feed to an adsorbent such as normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and desorbed using a mixture of polar and non-polar solvent. The source of the individual carotenes, tocols and sterols is obtained from vegetable oil or concentrate or phytonutrients rich-fractions. The preferred vegetable oil will be palm oil.

The said process is an isocratic and isobaric process wherein the operating pressure may range from 0.2 – 1000 bar. Solvents used for desorption may consist of non-polar solvents such as hexane, heptane, ethyl acetate, isooctane or cyclohexane and polar solvents such as ethanol, isopropanol, methanol or butanol.

It should be appreciated that the isolation of individual carotene, tocol and sterol by the said process can be carried out using the resulting fractions extracted from the concentrate or products from any sources that contains the said compounds.

Following is a description by way of examples of the recovery of carotenes, tocopherols, hydrocarbons such as squalene and sterols by different adsorbents.

Example 1

- 5 Crude palm oil methyl esters and palm oil minor components, derived from alcoholic esterification of crude palm oil with 1 to 2 % base catalyst and methanol is subjected to molecular distillation at a temperature less than 200°C and pressure not more than 150 mm Torr to give concentrate with composition of 5% carotene, 0.5% tocopherols, 0.7% squalene and 0.7% sterols.

10

- 1.4 gram sample of the above concentrate is dissolved in 10 milliliters of hexane and added to the top of a normal-phase silica gel packed column of 12cm long and 4cm internal diameter. The feed to adsorbent ratio is 23g/kg. Mixture of hexane and isopropanol with hexane:IPA (99.5:0.5) is added to the column in which it is applied
- 15 with not more than 1 bar pressure. Fractions are collected whereby squalene is the first to be eluted. This is followed by carotenes, tocopherols and last of all the phytosterols. Solvents in the collected fractions are rotavaporised. The concentration of squalene in the squalene fraction is determined by gas chromatography (GC) to be 7%, carotenes in the carotene fraction is determined by UV spectrometry at about 24%. The tocopherols are
- 20 determined by HPLC on silica 60A column having the dimension 250mm X 46mm I.D. with fluorescent detector. The mobile phase is hexane:THF:IPA (1000:60:4). The concentration of tocopherols in tocopherols fraction is about 62%. GC determines the composition of phytosterols and there are about 29% of sterols in sterols fraction.

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	7	24	62	29
Recovery %	92	41	3	5

Example 2

1.4 gram concentrate obtained from the same manner with the same composition as Example 1 is dissolved in 10 millilitres hexane and added to the top of a 12cm long, 4 cm internal diameter of normal-phased silica gel packed column. Mixture of Hexane:IPA (99.5:0.5) is then added to the column with an applied pressure of less than 1 bar. Fractions are collected and the concentration of the minor components in their respective fractions is determined to be: carotenes 28% with 87% recovery, tocols 71% with 38% recovery, and sterols 6%.

10

	Carotene	Tocols	Sterols
Concentration in feed, %	5	0.5	0.7
Concentration in fraction, %	28	71	6
Recovery, %	87	38	5

Example 3

2.5 gram of concentrate obtained from the same manner as in Example 1 is dissolved in hexane and added to the top of a 12cm long 4cm internal diameter normal-phase silica packed column. Mixture of hexane:ethanol (99.5:0.5) is added with pressure less than 1 bar to elute the minor components. Fractions collected are determined for carotenes and

tocols content. 43% carotenes are recovered from a fraction with 45% carotenes. In the tocols fractions, the concentration of the tocols is determined to be 100% with 8% recovery from that particular fraction.

	Carotene	Tocols
Concentration in feed, %	5	0.5
Concentration in fraction, %	45	100
Recovery, %	43	8

5

Example 4

0.5 gram concentrate obtained from the same manner as in Example 1 is added to the top of a 7.5cm long, 4cm internal diameter reversed-phase C18 silica gel packed column. Ethanol:hexane (60:40) are added to the top of the column with pressure less than 1 bar to elute the minor components. However, the range of ethanol:hexane can be changed depending on any other conditions up to 90:10. Sterols and tocols are first to be eluted and their concentrations are 4% and 3% each. The recovery of sterols is 72% and tocols is 75%. Carotenes and squalene are eluted in later fractions and the concentration of carotenes in their enriched fraction is 27% and the concentration of squalene is 4% with 40% recovery.

15

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	4	27	3	4
Recovery, %	40	40	75	72

Example 5

1.3g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter silica column. Mixture of hexane:ethanol (99.5:0.5) is added to the top of the column with pressure less than 1 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.7	5	0.5	0.7
Concentration in fraction, %	24	1	3	29
Recovery, %	34	3	2	58

Example 6

1.5g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter silica packed column. Mixture of hexane:IPA (99.5:0.5) is added to the top of the column with pressure less than 0.5 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	Carotene
Concentration in feed, %	0.1	7.9
Concentration in fraction, %	23	64
Recovery, %	71	60

The whole process uses less than 1.9 liters solvent and is completed in less than 30 minutes.

Example 7

- 5 1.6g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter C18 column. Mixture of Ethanol:Hexane (60:40) is added to the top to desorb the minor components with pressure less than 1 bar. Composition of carotene recovered is 14% with 12% recovery.

10 Example 8

- 2.9g concentrate rich in vitamin E is added to the top of a 12cm long, 40mm internal diameter silica column. Hexane:ethyl acetate (80:20) is added to elute the individual vitamin E with pressure less than 0.5 bar. α -tocopherol (α -T) is the first to be eluted followed by α -Tocotrienol (α -T₃), γ -Tocopherol (γ -T), γ -Tocotrienol (γ -T₃) and last of
- 15 all, the δ -Tocotrienol (δ -T₃). The purity of respective collected tocols are as follows:

	α -T	α -T ₃	γ -T	γ -T ₃	δ -T ₃
Composition in feed, %	9	9	1	16	3
Composition in fraction, %	42	54	9	68	40
Recovery, %	24	28	41	21	64

Example 9

2.5g concentrate rich in vitamin E is loaded to a 12cm long, 40mm internal diameter silica column. Hexane:Ethyl Acetate (86:14) is added to elute the vitamin E with pressure less than 0.5 bar. The purity of the respective tocopherols collected are as follows:

5

	α -T	α -T ₃	γ -T	γ -T ₃	δ -T ₃
Composition in feed, %	9	9	1	16	3
Composition in fraction, %	46	28	8	80	56
Recovery, %	33	22	32	30	49

Example 10

0.8g concentrate obtained from the same manner as Example 1 is dissolved in hexane and loaded to a 25cm long, 20mm internal diameter silica column. Mixture of hexane:IPA (98:2) is added to the column with pressure less than 1500psi to desorb the minor components. Squalene is the first to be collected followed by carotenes, vitamin E, sterols and last of all the unreacted diglycerides. The composition of the fractions are collected are as follows:

10

	Squalene	Carotene	Vitamin E	Sterols	Diglycerides
Composition in feed, %	1.5	2.6	1.9	0.9	2.6
Composition in fraction, %	4	50	18	3.4	20
Recovery, %	34	47	9	1	13

15

Example 11

1.6g concentrate obtained from the same manner as Example 1 is loaded into a 7cm long, 13cm internal diameter silica packed column. 100% hexane is added to the top to desorb the minor components with pressure less than 1 bar. First fraction collected consists of squalene while the second fraction consists of carotene. After the carotene fraction is collected, the polarity of the mobile phase is gradually increase using isopropanol started with 2 – 5% IPA in hexane. The later fraction collected consists of Vitamin E followed by sterols. The compositions and recovery of these fractions are as follows:

10

	Squalene	Carotene	Vitamin E	Sterols
Composition in feed, %	1.5	6.8	2.6	0.4
Composition in fraction, %	31	50	1.4	1.1
Recovery, %	80	39	26	25

Example 12

A 40mm I.D. column is packed with 6.6cm length normal phase silica and top up with 0.9cm acid treated Florisil. The silica and acid treated Florisil are separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. Feed to adsorbent ratio is 43g/kg. The concentrate consisted of 0.3% squalene, 3% carotene, 1% vitamin E, 0.6% sterols and 4% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Four fractions are collected.

The whole run took less than 20 minutes. The first fraction is colorless consisted of 34% squalene with 80% recovery. The second fraction is red in color consisted of 83% carotene with 53% recovery while the third fraction being orangey in color consisted of 3% vitamin E with 87% recovery. The last fraction is colorless consisted of 2% sterols.

- 5 After the last fraction is collected, residues in acid treated florisil are extracted using IPA. This residue consisted 30% monoglycerides.

Example 13

- A 40mm I.D. column is packed with 6.8cm length normal phase silica and top up with 0.7cm polyethylene glycol. The silica and polyethylene glycol are separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. The concentrate consisted of 0.4% squalene. 3% carotene, 4% vitamin E, 0.1% sterols and 5% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Five fractions are collected. The first fraction is colorless consisted of 30% squalene with 53% recovery. The second fraction is red in color consisted of 40% carotene with 80% recovery while the third fraction being orangey in color consisted of 10% vitamin E with 85% recovery. The fourth fraction is colorless consisted of 1% sterols with 35% recovery. The last fraction consisted of 75% diglycerides with 80% recovery. After the last fraction is collected, residues in polymer are extracted using IPA. This residue consists of 6% monoglycerides.
- 10
- 15
- 20

Example 14

A 40mm I.D. column is packed with 6.9cm length normal phase silica and top up with 0.6cm polyacrylate polyalcohol. The silica and polymer separated from each other with a layer of filter paper. 1.3 gram concentrate obtained from the same manner as Example 1 are added to the top of this column. The concentrate consisted of 0.4% squalene, 3% carotene, 4% vitamin E, 0.1% sterols and 5% monoglycerides. Hexane:IPA (99.5:0.5) are added to the top of the column to desorb the minor components with pressure less than 1 bar. Five fractions are collected. The first fraction is colorless consisted of 30% squalene with 70% recovery. The second fraction is red in color consisted of 34% carotene with 60% recovery while the third fraction being orangey in color consisted of 10% vitamin E with 60% recovery. The fourth fraction is colorless consisted of 0.7% sterols with 100% recovery and the last fraction consisted of 60% diglycerides with 90% recovery. After the last fraction is collected, residues in polymer are extracted using IPA. This residue consisted 30% monoglycerides.

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Example 15

1.3g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 7.5cm long, 4cm internal diameter silica column. Mixture of Hexane:IPA (99.5:0.5) is added to the top of the column with pressure less than 2 bar with flowrate 18ml/min to desorb the minor components. Fractions collected are determined with the following results:

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	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.2	2.6	1.0	0.8
Concentration in fraction, %	10	95	3	2.0
Recovery, %	93	96	84	21

Example 16

1.5g concentrate obtained from the same manner as Example 1 is dissolved in hexane and added to the top of a 12cm long, 4cm internal diameter silica column. Mixture of
 5 Hexane:IPA (99.5:0.5) is added to the top of the column with pressure at least 0.5 bar to desorb the minor components. Fractions collected are determined with the following results:

	Squalene	Carotene	Tocols	Sterols
Concentration in feed, %	0.2	4.2	1.0	0.8
Concentration in fraction, %	10	67	14.0	1.4
Recovery, %	100	42	60	41

Example 17

10 0.5g concentrate obtained from the same manner as Example 1 is dissolved in dichloromethane and loaded to a 25cm length, 20cm internal diameter RP C18 column. Mixture of acetonitrile:DCM (89:11) is added to the column with pressure less than 1500psi to desorb the carotenes. Four fractions are collected based on their respective UV-Vis absorption maxima. The first fraction with UV-Vis abs max at 500nm is 98%
 15 lycopene with 80% recovery. Second fraction consisted of α -carotene while the third

fraction consisted of β -carotene. The last fraction collected with UV-Vis abs max at 286nm is 95% phytoene with 80% recovery.

Example 18

5 0.2g sterols-rich fraction obtained from the first extraction is dissolved in methanol and loaded to a 25 cm length, 20 mm internal diameter RP C18 column. Mixture of MeOH:H₂O:CH₃COOH (98:1:1) is added to the column with pressure less than 1500psi to desorb the sterols. 5 fractions were collected in which the last fraction consisted of b-
10 sitosterol with more than 98% purity. Recovery of the said compound is >60%.

While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purpose of illustration, it will be apparent to those skilled in the art that the invention is
15 susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.

CLAIMS

1. A method for isolating minor components such as squalene, carotenes, tocopherols and sterols from a vegetable oil under an isocratic condition, said method comprising the steps of:

- 5 a) esterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components,
- b) collection of the esters phase containing the minor components from the glycerol;
- c) distillation of the esters phase, said distillation provides a concentrate of
10 squalene, carotenes, tocopherols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr;
- d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar;
- 15 e) adsorption of the concentrate obtained from step (d) on an adsorbent;
- f) extraction of the minor components from the concentrate obtained from step (e) by desorption of the minor components using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2
20 to 50 bar; and
- g) desorption of the minor components by a predetermined mixture of solvents.

2. A method as claimed in claim 1, wherein the vegetable oil is selected from palm oil.

3. A method as claimed in claim 1, wherein the alcohol in step (a) is selected from lower alcohols such as methanol, ethanol, isopropanol and butanol.
4. A method as claimed in claim 1, wherein the adsorbents used in step (e) is selected from normal-phase silica gel, reversed-phase (particularly C18) silica gel or neutral alumina and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.
5. A method as claimed in claim 1, wherein the solvent is selected from non-polar solvents such as hexane, heptane, dichloromethane, cyclohexane, petroleum ether, ethyl acetate, isooctane or cyclohexane.
6. A method as claimed in claim 1, wherein the solvent is selected from polar solvents such as ethanol, isopropanol, methanol, butanol or acetonitrile.
7. A method for isolating individual carotene such as β -carotene, α -carotene, lycopene, phytoene and phytofluene, individual tocol such as α -, γ - and δ -tocopherol and tocotrienol and individual sterol such as β -sitosterol from vegetable oil under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the method comprising the steps of:
 - a) adsorbing the oil to an adsorbent and
 - b) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

8. A method as claimed in claim 7, wherein the source of the individual carotene is concentrate or phytonutrients rich-fractions.

9. A method as claimed in claim 7, wherein the vegetable oil is selected from palm oil.

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10. A method as claimed in claim 7, wherein the adsorbent is selected from normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.

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11. A method as claimed in claim 7, wherein the non-polar solvent is selected from hexane, heptane, ethyl acetate, isooctane or cyclohexane.

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12. A method as claimed in claim 7, wherein the polar solvent is selected from ethanol, isopropanol, methanol or butanol.

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13. A method for isolating individual carotene such as β -carotene, α -carotene, lycopene, phytoene and phytofluene, individual tocol such as α -, γ - and δ -tocopherol and tocotrienol and individual sterol such as β -sitosterol from minor components such as squalene, carotenes, tocols and sterols obtained from a method which is conducted under an isocratic condition, the method comprising the steps of:

a) esterification of the oil with an alcohol, said esterification provides a mixture of glycerol, fatty acids esters and the minor components,

- b) collection of the esters phase containing the minor components from the glycerol;
- c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocopherols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr;
- d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar;
- e) adsorption of the concentrate obtained from step (c) on an adsorbent;
- f) extraction of the minor components from the concentrate obtained from step (d) by desorption of the minor components by a predetermined mixture of solvents which is a non-polar solvent or a mixture of a non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar; and
- g) desorption of the minor components by a predetermined mixture of solvents; characterised in that the minor components obtained from the above method undergo a further extraction under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the extraction comprising the steps of:
 - 1) adsorbing the minor components to an adsorbent and
 - 2) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

14. A method as claimed in claim 13, wherein the vegetable oil used in step (a) is selected from palm oil.

15. A method as claimed in claim 13, wherein the adsorbent for step (1) is selected from
5 normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.

16. A method as claimed in claim 13, wherein the non-polar solvent for step (2) is
10 selected from hexane, heptane, ethyl acetate, isooctane or petroleum ether.

17. A method as claimed in claim 13, wherein the polar solvent for step (2) is selected from ethanol, isopropanol, methanol, butanol ethyl acetate or acetonitrile.

A METHOD OF EXTRACTING AND ISOLATING MINOR COMPONENTS FROM VEGETABLE OIL

ABSTRACT

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A process for the separation and recovery of minor components from vegetable oil such as palm oil. Esterified palm oil is subjected to molecular distillation giving a concentrate rich in minor components. The concentrate is then adsorbed to adsorbents such as normal phase silica gel, reversed phase (particularly C18) silica gel or neutral alumina as well as polymer absorbents such as polyethylene glycol and polyacrylate polyalcohol. Minor components such as squalene, carotenes, tocopherols and sterols are then selectively desorbed, separated and recovered using solvents operating at isocratic conditions. The desorption of the said minor components are sped up by applying pressure of between 0.2 to 50 bar. By the same method, individual tocopherol isomers from palm oil can also be separated and recovered.

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